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A Review on Tannase and its Applications

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Abstract

Tannin acyl hydrolase (E.C. 3.1.1.20) or Tannase is a type of hydrolase enzyme that catalyzes the hydrolysis of tannin and is converted to gallic acid and glucose. Natural tannins occur in various parts of plants such as leaves, roots, bark and fruits. Tannins are distributed in animals, insects and microorganisms. However, microbial tannase is one of the great attention in many industries. Gallic acid is one of the industrial and therapeutic molecules that is widely used in various applications. Tannase is also applicable in feed, pharmaceutical, brewing, chemical and beverage industries. Furthermore, tannase plays a significantly important role in biodegradation or reduction of tannin from the tannery effluent. The present review apprises a brief description of tannase and its industrial applications.

Keywords: Tannin; Tannase; Gallic acid; Glucose; Tannery effluent.

Introduction

Tannases represent a group of enzymes, that are produced by microbes, plants and animals. However, tannase from microbial sources is preferred over other sources for industrial. The catalytic reaction of tannase involves the hydrolysis of ester bond and depside bond present in various substrates i.e., tannic acid, complex tannin, gallic acid esters, epigallocatechin gallate, methyl gallate, ethyl gallate, n-propyl gallate to produce gallic acid (3, 4, 5-tri hydroxybenzoic acid) and glucose. Various parameters such as temperature, pH, metal ions, activators, inhibitors and substrate specificity affect the enzyme activity.

Tannase enzymes are used in the food, brewing, and pharmaceutical industries. Gallic acid production is one of the most important commercial applications of tannase. Apart from that, they are extensively used in the food industry, especially in instant tea production, where it enhances the extractability and cold water solubility of key compounds. Another important application of tannase is the removal of haze formation and unflavored phenolic compounds from beer and wine and fruit juices. The quality of fruit juices also can be improved by the tannase enzyme.

Industries require novel microbial strains for enzyme production using easily available inexpensive raw materials and trying to evolve various dimensions of tannase such as novel sources of tannase, new microbes with high tannin transformation ability, new substrate specificities, low cost production, less expenditure on the purification strategies and the potential use of tannase in different industries has been shown by researchers through out the world. The current review articles explain in detail the structure, purification and application of tannase produced by microbes.

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Tannins

The meaning of Tannin or Tanna (German word) is oak, which refers to the use of tannins from the wood/bark of oak or fir trees that were used in the tanning process (Barbehenn and Constabel, 2011; Tomak and Gonultas, 2018). Tannins are well known large, complex, natural plant derived polyphenolic biomolecules that are found in the bark, stem, buds, wood, leaves, fruits and roots. Tannin plays a very important role in protecting plants from microorganisms and other predators (Khanbabaee and van Ree, 2001; Sharma, 2019). In a few countries, tannin rich plant extracts serve as traditional medicines for the treatment of cancer, diarrhea, inflammation, dieresis, hemorrhagic stroke and skin (Dai, 2010 and Działo, 2016).

Based on diversified structures and properties of tannins, they are classified into three major classes and two subclasses: (1) Hydrolysable tannins (2) Condensed tannins and (3) Complex tannins (Figure 1). Hydrolysable tannins can be defined as the types of tannins that are hydrolysed into gallic acid and ellagic acid in the presence of acid or alkaline conditions, hot water and enzymes. Hydrolysable tannins are further classified into two subclasses based on their hydrolysable products i.e. (i) Gallotannins and (ii) Ellagitannins. Hydrolysable tannins are mostly found in berries, grapes, nuts, coffee, tea, fruits and wine. The second class is condensed tannin or non-hydrolysable tannin. They are flavanoid tanning substances that belong to oligomeric and polymeric proanthocyanidins. Condensed tannins are obtained from cocoa, various fruits like pears, apples, green grapes, legumes such as chickpeas, red kidney beans and peas, respectively (Costain, 2001; Selma et al.,2009). The third class of tannin is complex tannin. Complex tannins or non-classified tannins have catechin units that are glycosidically bound to the gallic acid (gallotannins) or ellagic acid (ellagitannins). Several sources of complex tannins are camelliatannins A and B (Camellia japonica L), malabarin A (Melastoma malabathricum) and acutissimin A. (Okuda and Ito, 2011).

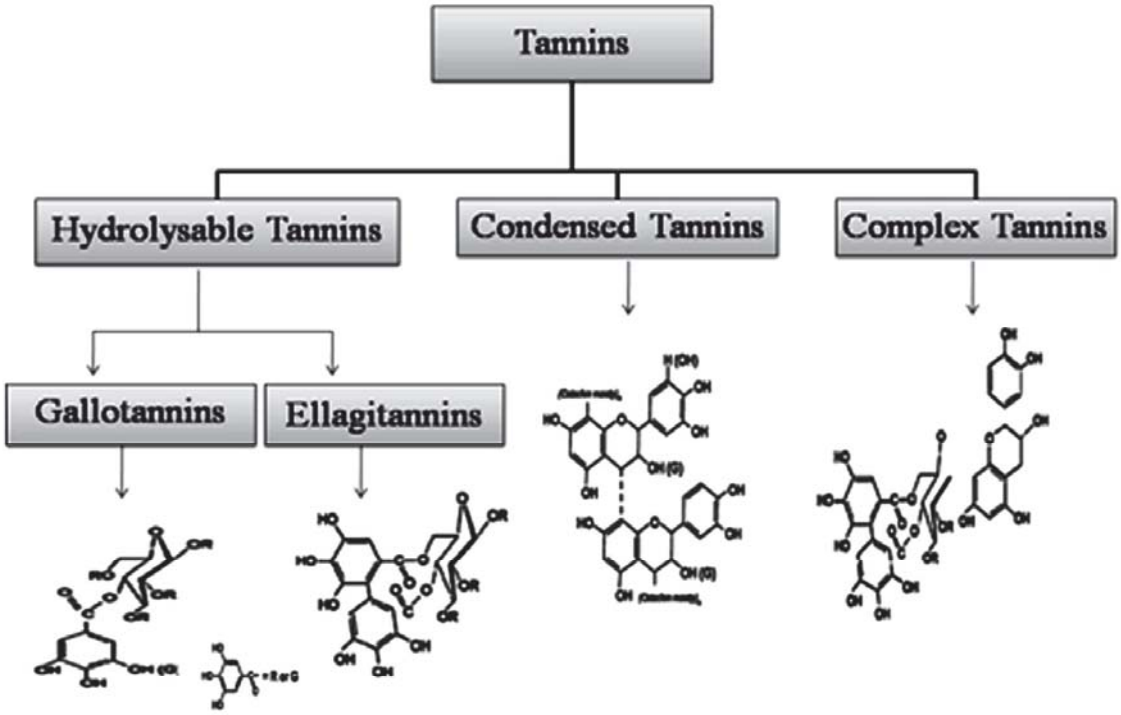


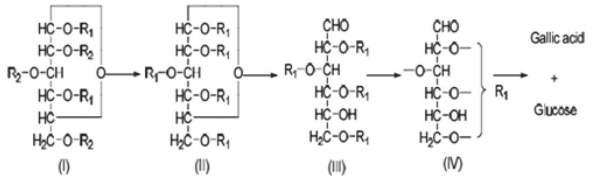
Fig. 1: Major Classes of Tannins.

Hydrolysable tannins can be helpful in the leather, wine and plastic manufacturing industries (Guo et al.,2020; Motta et al.,2020). They can be used as antimutagenic, anticancer, antioxidant, wood preservative, anti-corrosion agents for metals. (Tomak and Gonultas, 2018; Byrne et al., 2019).

Tannase

Tannase (E.C. 3.1.1.20) or tannin acyl hydrolase (TAH) is an explored group of hydrolases family. The catalytic reaction of tannase involves the hydrolysis of ester bond and depside bond present in various substrates i.e., tannic acid, complex

tannin, gallic acid esters, epigallocatechin gallate, methyl gallate, ethyl gallate, n-propyl gallate to produce gallic acid (3, 4, 5-tri hydroxybenzoic acid) and glucose (Lal and Gardner, 2012). Several intermediate molecules (Figure 2) are formed during the hydrolytic breakdown of tannic acid into gallic acid and glucose by tannase enzyme i.e (II) 1, 2, 3, 4, 6, - pentagalloyl glucose, (III) 2,3,4,6 tetragalloyl glucose and (IV) monogalloyl glucose (Lekha and Lonsane, 1997).



(R1-gallic acid; R2–m-di gallic acid; I-tannic acid, II-1,2,3,4,6-pentagalloyl glucose; III - 2,3,4,6 tetragalloyl glucose and IV- monogalloyl glucose)

Fig. 2: Mechanism of hydrolysis of tannic acid by Tannase (Lekha and Lonsane, 1997)

The novel biotechnological approach of gallic acid is the production of trimethoprim. Gallic acid has emerged as a basic intermediate of trimethoprim containing bacteriostatic and broad-spectrum features. (Anderson et al., 1980; Misro et al., 1997; Mukherjee and Banerjee, 2003). The enormous applications of gallic acid such as in the manufacture of ink, dye and paper,in photographic development, in the tanning process of leather are found in many sectors of various industries.

Tannase Structure

Ren et al. (2013) reported the monomeric three-dimensional structure of tannase from Lactobacillus plantarum SICC 1.15 (Figure 3). This tannase contains 50,747 Da molecular weight and 469 amino acid residues. However, the crystal structure of tannase consists of two domains such as α/β-hydrolase domain (residues 4-204 and 396-469) and lid domain (residues 205-395). The α/β-hydrolase domain contains six α helices such as α1-α4 and α 12- α13 and nine β-stranded sheets such as β1-β7 and β 12-β13. Among the α helices, α2- α4 and α12 are present on one side of the sheet, while α1 and α13 are present on the opposite side. The other domain known as the lid domain contains seven α-helices such as α5- α11 and two β-stranded sheets such as β 8- β9 and β10-β11. The two β-stranded sheets lie between the β 7-12 strands. The active site of the tannase enzyme is revealed in the α/β-hydrolase domain. A deep tunnel is formed by the juxtaposition of α/β-hydrolase and lid domain. The tunnel wall is formed by three amino acid residues as Ser163, Asp419 and His451, known as a catalytic triad. Along with the catalytic triad (Ser163, Asp19 and His451), tannase also contains serine residue Ser163 that is located in the pentapeptide motif i.e.GXSXG or Gly161-X-Ser163-X-Gly165 (where X = any amino acid residue) between β6 and α6. The active site of the tannase consists of three charged residues as Lys 343, Glu357 and Asp421. These three charged residues play a major role in the hydrolysis and binding of galloyl moiety of the substrate.

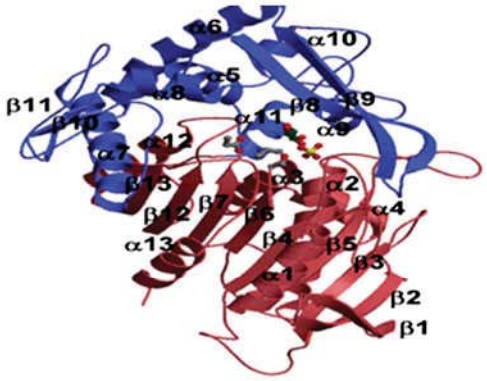


Fig. 3: Structure of L. plantarum tannase. The structure contains α/β- hydrolase domain in red ribbon view and lid domain in blue-ribbon view (Matoba et al.,2013)

Sources of Tannase

Tannase can be found in prokaryotic and eukaryotic cells. It is widely distributed in animals, plants and microorganisms. Among various sources, microbial strains play a crucial role in tannase production because of their great stability over plants and animals. Bacteria, molds and yeasts produce 30%, animals produce 8% and plants produce 4% industrial enzymes.

Plants derived tannase

Tannin-rich plants are great sources for tannase production. Various parts of plants such as bark, woods, stems, roots, fruits, leaves and seeds contain a high amount of tannin. Different plant species like Acacia katechu, Agrimonia eupatoria, Diospyros kaki, Hamamelis virginiana, Monochaetum multiflorum, Syzygium cumini, Terminalia chebula and Quercus robur were reported to be efficient tannase producers (Shen et al.,2006; Granica et al.,2013; Zhange et al., 2011). Plants produce a variety of acids such as chebulinic acid, gallic acid, and hexahydroxyphenic acid and a large quantity of sugar, during the growth phase. As fruits become mature/ ripen, tannase esterified these acids with sugar (i.e glucose) and form complex tannin (Lekha

and Lonsane, 1997).

Animals and Insects Derived Tannase

The ruminal mucosa of several animals such as koalas (Phascolarctos cinereus) (Osawa, 1992), goats (Capra hircus) (Brooker et al., 1994; Nelson et al., 1995), and horses (Equus caballus) (Nemoto, 1995) contain a small amount of tannase that degrade tannin from the nutritional resources and narrow down harmful effects of tannin on animals (Mosleh et al., 2014). Tannase can be extracted from the larva of some insects such as Indian Mole Cricket (Gryllotalpa krishnani), Cynipid, Salix caprea (Mani, 1966). The larva of the insects contains other digestive enzymes such as invertase and diastase. The role of digestive enzymes is to act on the large molecules of plant cells and convert them into simple forms and help insects in digestion. Tannin present in the leaves of plants precipitates with digestive enzymes and inactivates them. Tannase from the larva of insects acts on tannin and protects the digestive enzymes (Mani, 1966).

Microbially derived tannase

Many microorganisms such as bacteria, fungi, yeast are known to be tannase producers. Tannase producing microbes utilize tannin as a sole source of carbon and transform tannin into glucose and gallic acid.

Bacterial tannase

For the past few decades, the most significant approach to obtain tannase is from bacterial source compared to yeast and fungal sources due to its high genetic manipulation, biochemical diversity, stability in an extreme environment, and extracellular enzyme production (Jana et al., 2014, Aguilar and Gutierrez Sanchez, 2001). In earlier reports, many bacterial strains such as Achromobacter, Klebsiella, Bacillus, Corynebacterium., Citrobacter, Streptococcus, Leuconostoc Pediococcus, Pantonea and Streptomyces were able to produce tannase. Out of 21 different bacterial genera, Lactobacilli are the most dominant tannase producing bacterial genera among others (Chandrasekaran and Beena, 2013).

Hydrolysable tannins such as gallotannins and ellagitanins are hydrolysed by bacterial tannase (Bhat et al., 1998). Glucose and gallic acid are the hydrolysed products of tannin through tannase. Gallic acid is decarboxylated by the enzyme gallate decarboxylase and converted to pyrogallol. The pyrogallol is converted into pyruvic acid, cis- aconitic acid and 3-hydroxy-5-oxo-hexanoate (Figure 4). These three molecules enter and circulate into the TCA cycle. 3-hydroxy-5-oxo-hexanoate is the breakdown product of dihydro phloroglucinol. Dihydro phloroglucinol and phloroglucinol are produced through catalytic actions of diphloroglucinol hydrolase and phloroglucinol isomerase from pyrogallol.

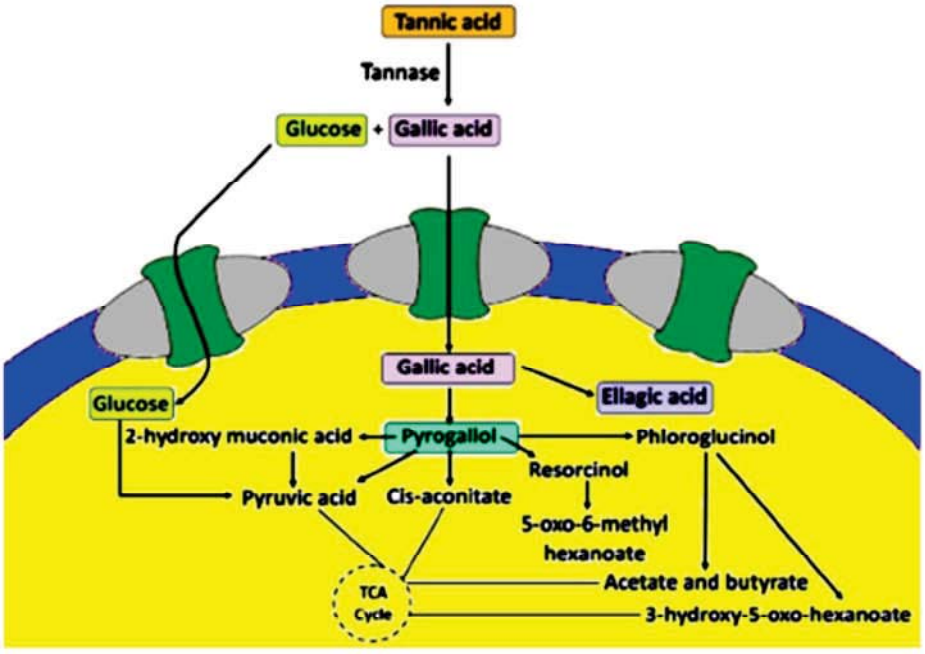


Fig. 4: Pathway showing Tannin degradation by bacterial tannase (Jana et al., 2014)

Fungal tannase

Fungal tannase have better degrading ability for hydrolysable tannin and condensed tannin (Belmares-Cerda et al., 2003). Out of 20 different genera of fungi, Aspergilli and Penicillia are the most dominant groups of tannase producers (Raghuwanshi, 2012). Among 2o different fungal genera, 27 species of Aspergillus, 24 species of Penicillium, 4 species of Trichoderma and 3 species of Fusarium are known as tannase producers. Several fungal species such as Mucor sp., Paciliomyces sp., Hyalopus sp. and Neurospora sp. are also reported as tannase producers. The pathway of tannin degradation by fungal tannase is shown in Figure 5.

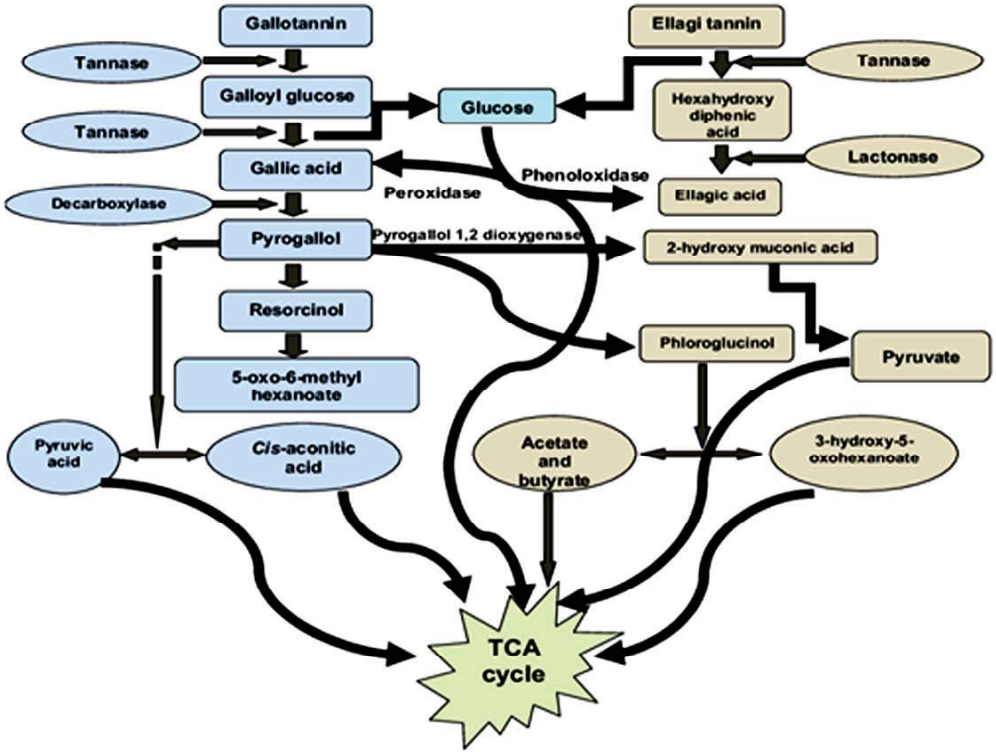


Fig. 5: **Pathway showing Tannin degradation by fungal tannase (Banerjee and Mahapatra, 2012)**

Yeast Tannase

Yeast tannases are less efficient for degrading naturally obtained tannin compare to tannic acid (Deschamps et al.,1983). Nowadays, researchers develop conventional and biotechnological approaches for the production of tannase from yeast due to its short growth period and easy accessibility towards molecular genetics. Various yeast species such as Candida sp., Pichia sp., Debaryomyces hansenii, Mycotorula japonica and Saccharomyces cerevisae are reported as tannase producers.

Actinomycetal Tannase

Various actinomycetal species such as Streptomyces sviceus (Wang,et al.,2018), Streptomyces gancidicus (Tripathi et al., 2021), Streptomyces sp. AL1L (Roy et al., 2018) are reported as tannase producers.

Tannase Assay

Diversified analytical methods such as UV-spectrometric (Parmentier, 1973 and Iibuchi et al.,1967), colorimetric (Haslam and Tanner, 1970), photometric (Chen,1969), titrimetric (Freudenberg et al., 1927; Nishira, 1961; Haslam and Stangroom, 1966), chromatographic (Jean et al., 1981) etc. are reported. All these methods used for assay of tannase are based on either determination of residual tannic acid (Deschamps et al., 1983) or formation of gallic acid, a byproduct of enzymatic hydrolysis of tannic acid.

Solid state and Submerged Fermentative production of tannase

Fermentation is the metabolic process in which complex compounds are converted into simple compounds by using various microorganisms such

as bacteria and fungi. The fermentation process is classified into two broad classes as solid-state fermentation (SSF) and submerged fermentation (SmF) based on the type of substrates used in the process.

Solid-state fermentation or SSF is defined as a process in which microbes utilize natural substrates or non-soluble inert supports as a nutritional source and can grow in the presence of low water availability or absence of water. SSF is a favorable method for tannase production due to its simple operating system, less water consumption and low-cost production (Aguilar et al., 2001 and Barrios González, 2012). Selwal et al. (2010) found maximum tannase activity in the presence of amla (Phyllanthus emblica) and keekar (Acacia nilotica) leaves by Pseudomonas aeruginosa IIIB. Ding et al. (2020) used mixed strains of Bacillus subtilis, Aspergillus niger and Saccharomyces cerevisiae for the production of tannase during solid-state fermentation of tea residue.

Submerged fermentation or SmF is defined as a process in which microbes utilize dissolved or suspended nutrients from the liquid medium (Frost, 1987). Among the various fungi, Penicillium verrucosum and Aspergillus niger degraded tannin from the coffee pulp and tea residue, respectively under SSF and produced tannase (Bhoite and Murthy, 2015 and Sharma et al.,2014). Submerged fermentation is more preferable comparedd to solid-state fermentation for the production of bacterial and fungal tannase in various industries. Bacillus megaterium showed maximum tannase activity (10.77 U/mL/ min.) in the presence of 1% tannic acid concentration under SmF (Tripathi et al.,2016).

Tannase Purification

Most of the produced enzymes are extracellular or intracellular through the fermentation process. Cells are directly separated from the fermentation broth for the recovery of the extracellular enzyme, wherein the case of intracellular enzyme cells are disrupted by the mechanical or non mechanical methods. The purification process mostly depends upon the need for the enzyme in the market. Purification of the enzyme is carried out to recover a high yield with the greatest catalytic activity and purity. Downstream processing is essential to remove the impurities from the end product. It is a multistep process in which various methods such as Salting out or solvent precipitation, Ultrafiltration, electrophoresis and chromatography methods could be employed. The initial step for purification of an enzyme is ammonium sulphate precipitation. The role of ammonium sulphate in purification is to stabilize the protein. Various types of precipitating agents like 2 ethoxy 6-9 diamino acridiniiun lactate or rivanol, as well as such polymers like dextran, PEG (polyethylene glycol) and polyvinyl alcohol, were used for the precipitation of tannase (Sharma et al., 1999 and Aoki et al., 1976). The second step of enzyme purification is a desalting process or dialysis. Dialysis is a process in which small molecules are removed from a mixture of molecules. The process was performed by using a semipermeable membrane. The third step applicable in tannase purification is ion exchange chromatography. The protein separated by ion-exchange chromatography is based on its charge that interacts with an oppositely charged ion-exchange matrix. Many researchers have used anion exchangers such as DEAE-Sephadex, DEAE-cellulose, DEAE-Sephadex A-50, and DEAE Sepharose for purification of tannase due to its acidic nature (Bhardwaj et al., 2003, Mahendran et al., 2006, Sharma et al., 1999). The fourth or last step of enzyme purification is gel permeation or size exclusion chromatography. The gel beads used in gel filtration chromatography are prepared from Sepharose, Agarose, Vinyl polymers, Polyacrylamide and Sephadex. Sephadex G-100, Sephadex G-200 and Sephadex G-150 were used as a gel in gel filtration chromatography for tannase purification (Beena et al.,2010, Ramirez–Coronol et al.,2003 and Sharma et al., 1999).

Biochemical Properties and Kinetics of Tannase

Different tannases obtained from different microbial sources are characterized and have some biochemical properties that give information related to the nature and structure of the enzyme. Various parameters such as temperature, pH, metal ions, activators, inhibitors and substrate specificity affect the enzyme activity. Microorganisms express the gene for the enzyme at specific pH. The optimum fungal and bacterial tannase biosynthesis has been reported in 3-8 pH range. However, in certain reports, the optimum pH of 8 and 8.9 was reported for bacterial tannase (Belur et al.,2010 and Matsuda et al., 2016). The optimal pH for actinomycetal and yeast tannase was 6 and 4.5, respectively (Roy et al., 2018 and Pan et al., 2020). Tannase from A. fumigates was stable at pH 4.0 and it lost enzyme activity at pH 8.0 (Batra and Saxena, 2005). Mahapatra et al. (2005) reported that mostly fungal tannase required an acidic environment to become active. Many researchers reported the optimum temperature range of some tannase-producing microorganisms

was between 30-70 °C (Raghuwanshi et al., 2011; Beniwal et al., 2013 and Govindarajan et al., 2018). The native structure of tannase might be affected by the presence of metal ions due to an increase in the ionic strength of the solution (Chaitanyakumar and Anbalagan, 2016). At a high concentration of metal ions, tannase activity was decreased. In the previous kinds of literature, tannase activity was enhanced in the presence of sodium chloride (Mondal et al., 2001; Aftab and Hamid, 2016). The catalytic activity of the enzyme is affected by the surfactants (Prasad et al., 2012). Sodium lauryl sulfate (SDS) showed the stimulatory effect on tannase produced from B. subtilis at 1% (v/v) concentration, while Tween 80, Tween 60, DMSO (Dimethyl Sulfoxide), Sodium azide, β-mercaptoethanol, EDTA, PMSF (phenylmethylsulfonyl fluoride) and Triton X-100 showed a negative effect on tannase activity (Jana et al., 2013). Various polar and nonpolar organic solvents also affect tannase activity. Several nonpolar solvents such as hexane, toluene and benzene enhanced tannase activity. However, few polar solvents such as methanol, isoamyl alcohol and butanol inhibited tannase activity (Beniwal et al., 2010; Valera et al., 2015).

Applications of Tannase

Tannase is an important industrially relevant and versatile enzyme that has tremendous applications such as in the manufacturing of tea, wine, beer, food, pharmaceutical, chemical, brewing and leather industries. Moreover, the tannase enzyme is also used for the bioremediation of toxic wastes from tannery effluents.

Gallic acid Production

Gallic acid or 3, 4, 5-trihydroxybenzoic acid is an organic compound that is widely used in pharmaceutical and chemical industries. Gallic acid is obtained from various sources such as plants and microorganisms. Gallic acid is present in a plant as free molecules or it can be produced from the tannase-producing microorganisms by hydrolysis of tannic acid or tannin-rich substrates. 3, 4, 5 trimethoxy benzaldehyde is the intermediate component during the manufacturing of trimethoprim from gallic acid. Trimethoprim is effective against gram-negative and gram-positive microorganisms when it synergistic with sulfonamide. Trimethoprim inhibits the dihydrofolate reductase enzyme and prevents the synthesis of folic acid. Folic acid plays a very important role in the synthesis of DNA. Gallic acid serves as an antiapoptotic, antiviral, antimutagenic and anticancer agent (Badhani et al., 2015).

Instant tea Preparation and Tea Cream Solubilization

Tea is the most highly consumed and favorable beverage worldwide, after water. There are so many health benefits of tea such as it helps to reduce the risk of cancer, heart disease and diabetes. When tea leaves are treated with tannase. It improves the flavor of tea with better acid stability and cold water solubility (Natarajan et al., 2009). It also enhances the bright reddish color due to the formation of epitheaflavic acid from epicatechin and gallic acid. Haze and tea cream formation during the preparation of tea beverages should be removed by tannase treatment.

In Beverages

The application of tannase in beverage industries is to remove water insoluble precipitates and undesirable tannin which is responsible for haze formation and bitter taste (Boadi and Neufeld, 2001). Additionally, tannase plays a major role in the removal of tannins and phenolic compounds from the different fruit juices and influences the flavor and maintains the color stability of the juice during the long period of preservation (Aguilar et al., 2007).

In Animal feed and Cell wall Digestion

The quality of animal feed can be also improved by the digestibility of the plant cell wall. Tannase acts on crosslinks of plant cell wall polymers that contain ferulic acid dehydrodimers. Tannase hydrolyzes diethyl diferulates and improves the quality of animal feed (Garcia-Conesa et al., 2001).

In the Biological Treatment of Tannery Effluent

The tannery industry is one of the largest industries and most important for the tanning of leather in India. The tanning process is necessary to prevent the leather from decomposition and to impart the color of the leather. Moreover, it causes environmental pollution during the discharge of tannery wastes to sewage or natural bodies. Besides these, tannin present in the effluent also inhibits the microorganisms. Various chemical and biological methods are used to remove tannin and other phenolic compounds from the tannery effluent. Recently, biodegradation technology is widely used for the treatment of tannery effluent and other types of industrial wastes. Tannase treatment is an eco-friendly treatment that helps to remove tannin and decrease pollution load such as BOD and COD from industrial wastes or wastewater (Zhao et al., 2017).

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